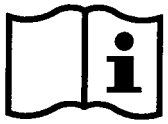
Product information



Userś Manual

Sphingomyelin IgG/M ELISA

Enzyme Immunoassay for the detection of IgG and/or IgM antibodies directed against Sphingomyelin in human serum.

REF AE29008 Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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Instruction Manual

Table of Contents

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1 INTENDED USE

This Sphingomyelin IgG/M ELISA is a solid phase enzyme immunoassay with highly purified sphingomyelin and native human β2-glycoprotein I for the quantitative and qualitative detection of IgG and/or IgM antibodies against sphingomyelin in human serum. These antibodies recognize specific epitopes on a complex composed out of sphingomyelin and β2-glycoprotein I.

For research use only, not for use in diagnostic procedures.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

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2 KIT CONTENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Containing: Immunoglobulins conjugated to horseradish peroxidase
Conjugate, IgM	1 x 15ml	Green	Green	Containing: Immunoglobulins conjugated to horseradish peroxidase
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

3 STORAGE AND SHELF LIFE

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

4 PRECAUTIONS OF USE

4.1 Health Hazard Data

THIS PRODUCT IS FOR RESEARCH USE ONLY. Thus, only staff trained and specially advised in method of ELISA techniques may perform the kit. **NOT FOR USE IN DIAGNOSTIC PROCEDURES.** Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by the CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All biological source material used for some reagents of this kit has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle these as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

4.2 General directions for use

In case that the product information, including the labelling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32ºC/68-89.6ºF) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommendd test performance at 30ºC/86ºF for automated systems.

Never expose components to higher temperature than 37ºC/98.6ºF

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

5 SAMPLE COLLECTION AND PREPARATION

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8 hours, respectively stored tight closed at 2-8ºC/35-46ºF up to 48 hours, or frozen at -20ºC/-4ºF for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

6 ASSAY PROCEDURE

6.1 Preparation prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20ml plus 80ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20ml plus 980ml).

To avoid mistakes, we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000µl sample buffer (1x) + 10µl serum. Mix well!

Washing:

Prepare 20ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells.

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual Washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean absorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8ºC/35-46ºF).

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6.2 Pipetting Scheme

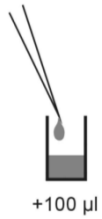
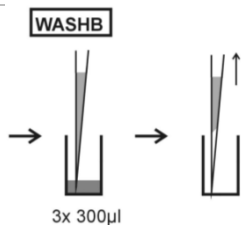
WE suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

Cal.A: calibrator A	Cal.D: calibrator D	PC: positive control	P1: sample 1
Cal.B: calibrator B	Cal.E: calibrator E	NC: negative control	P2: sample 2
Ca.C: calibrator C	Cal.F: calibrator F	CC: cut-off calibrator	P3: sample 3

6.3 Test Steps

Step	*Description*
1.	Ensure preparations from step 6.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative interpretation results desired:
	CONTROLS & SAMPLES
3.	 Pipette into the designated wells as described above, 100 µl of either: Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • diluted serum (P1, P2...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x with 300 µl washing buffer (diluted 1:50).

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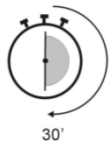
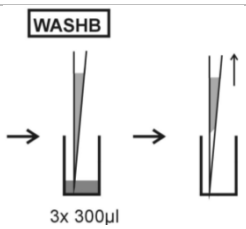
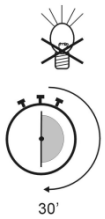
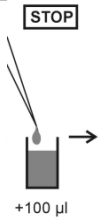
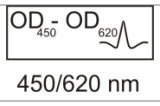
	CONJUGATE	
6.		Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.		Wash 3x with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

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7 INTERPRETATION

For **quantitative interpretation** establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve
Do not use this example for interpreting your results!

Calibrators	OD 450/620 nm	CV % (Variation)
0 U/ml	0.039	0.0
3 U/ml	0.156	1.4
10 U/ml	0.310	2.1
30 U/ml	0.615	1.8
100 U/ml	1.253	2.9
300 U/ml	2.091	0.8

Example of calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.841/1.833	1.834	219.4
P 02	0.596/0.563	0.580	27.1

For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and sample population according to their own established procedures.
In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

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8 TECHNICAL DATA

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

9 LITERATURE

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Boey, M.L., Colaco, C.B., Gharavi, A.E., et al. (1983). Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. Br. Med. J. 287: 1021-1023.

Gastineau, D.A., Kazmier, F.J., Nichols, W.L., Bowie, E.J. (1985). Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases. Am. J. Hematol. 19: 265-267.

McNeil HP, Simpson RJ, Chesterman CN, Kirilis SA (1990). Anti-phospholipid antibodies are directed against a complex antigen that includes a lipuid-binding inhibitor of coagulation: ß2-Glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 87: 4120-4124.

Wöhrle R, Matthias T, von Landenberg P, Oppermann M, Helmke K, Förger F (2000). Clinical relevance of antibodies against different phospholipids. Journal of Autoimmunity 15: A60.

Lothar Thomas: Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books

CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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